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Review

Formin-binding proteins: Modulators of formin-dependent actin polymerization

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ABSTRACT

Formins represent a major branch of actin nucleators along with the Arp2/3 complex, Spire and Cordon-bleu. Formin-mediated actin nucleation requires the formin homology 2 domain and, although the nucleation *per se* does not require additional factors, formin-binding proteins have been shown to be essential for the regulation of formin-dependent actin assembly *in vivo*. This regulation could be accomplished by formin-binding proteins being directly involved in formin-driven actin nucleation, by formin-binding proteins influencing the activated state of the formins, by linking formin-driven actin polymerization to Arp2/3 driven actin polymerization, or by influencing the subcellular localization of the formins. This review article will focus on mammalian formin-binding proteins and their roles during vital cellular processes, such as cell migration, cell division and intracellular trafficking.

1. Introduction

The ability of eukaryotic cells to undergo dynamic reconstructions of their shape is a prerequisite for cellular processes, such as cell migration and cell division. These vital processes are dependent on the perpetual reconstruction of cytoskeletal elements and the actin filament system is of particular importance for cell migration and the maintenance of cell shape. In order to ensure a coordinated spatial and temporal organization of the actin filament system, nature has provided cells with at least four machineries for actin assembly: the Arp2/3 complex, formins, Spire (Spir) and Cordon-bleu (Cobl) [1,2]. The Arp2/3 complex and the formins appear to be universal actin nucleators that seem to be expressed in most cell-types, whereas Spir and Cobl have a more cell-type specific expression pattern, e.g. the proteins are abundant in the brain [1,2].

In order to catalyze the nucleation of actin filaments, the Arp2/3 complex requires additional proteins, such as cortactin or members of the Wiskott–Aldrich syndrome protein (WASP)/WASP family verprolin homologous (WAVE) family of proteins [3]. In contrast, the formins can nucleate actin in the absence of additional factors solely by virtue of their formin homology 2 (FH2) domains [1,4,5]. Studies from many laboratories have established the existence of several categories of formins but the presence of the FH2 domain is the common denominator to the formin family of proteins [4–7]. The FH2 domain is almost always accompanied by the proline-rich formin homology 1 (FH1) domain located N-terminally to the FH2 domain (Fig. 1A). The *formin* gene was originally cloned as the candidate gene responsible for limb deformity in mice [8]. However, later work showed that *formin* ablation was not causing limb deformity, instead an adjacent gene, *gremlin*, a bone morphogenetic protein antagonist, was shown to be responsible for the phenotype in mice [9]. Despite the questionable origin of the name, formin, it has stuck as the common designation of this family of proteins.

Most eukaryotes have multiple formin genes and plants harbor over 20 different formins, indicating that FH2-driven actin nucleation is a fundamental process in all eukaryotic cells (Table 1) [6,7]. Mammalian cells encode at least 15 formins, which can be divided into 2 subfamilies, formins and Diaphanous-related formins (DRFs) (Fig. 1A). Most of our current knowledge of the mammalian formins stems from studies on DRFs [4,5]. The DRFs exhibit several distinct domains in addition to the FH1 and FH2 domains; the GTPase binding domain (GBD) in the N-terminus and the Diaphanous autoregulatory domain (DAD) in the C-terminus. The GBD binds members of the Rho family of small GTPases but it also contains motifs for binding to the DAD. The formin homology 3 (FH3) domain, which is a less well defined domain rich in coiled-coil motifs, resides between the GBD and the FH1 domain and it is important for dimerization (Fig. 1A) [4,5]. According to the current paradigm, the DRFs in resting cells reside in an autoinhibitory conformation, mediated by an interaction between the DAD and a part of the GBD domain. Signaling cues, provided by formin-binding proteins (FBPs), such as activated Rho GTPases, will result in the release of the autoinhibited conformation (Fig. 1B) [4,5]. The Rho GTPases thus constitute an important group of

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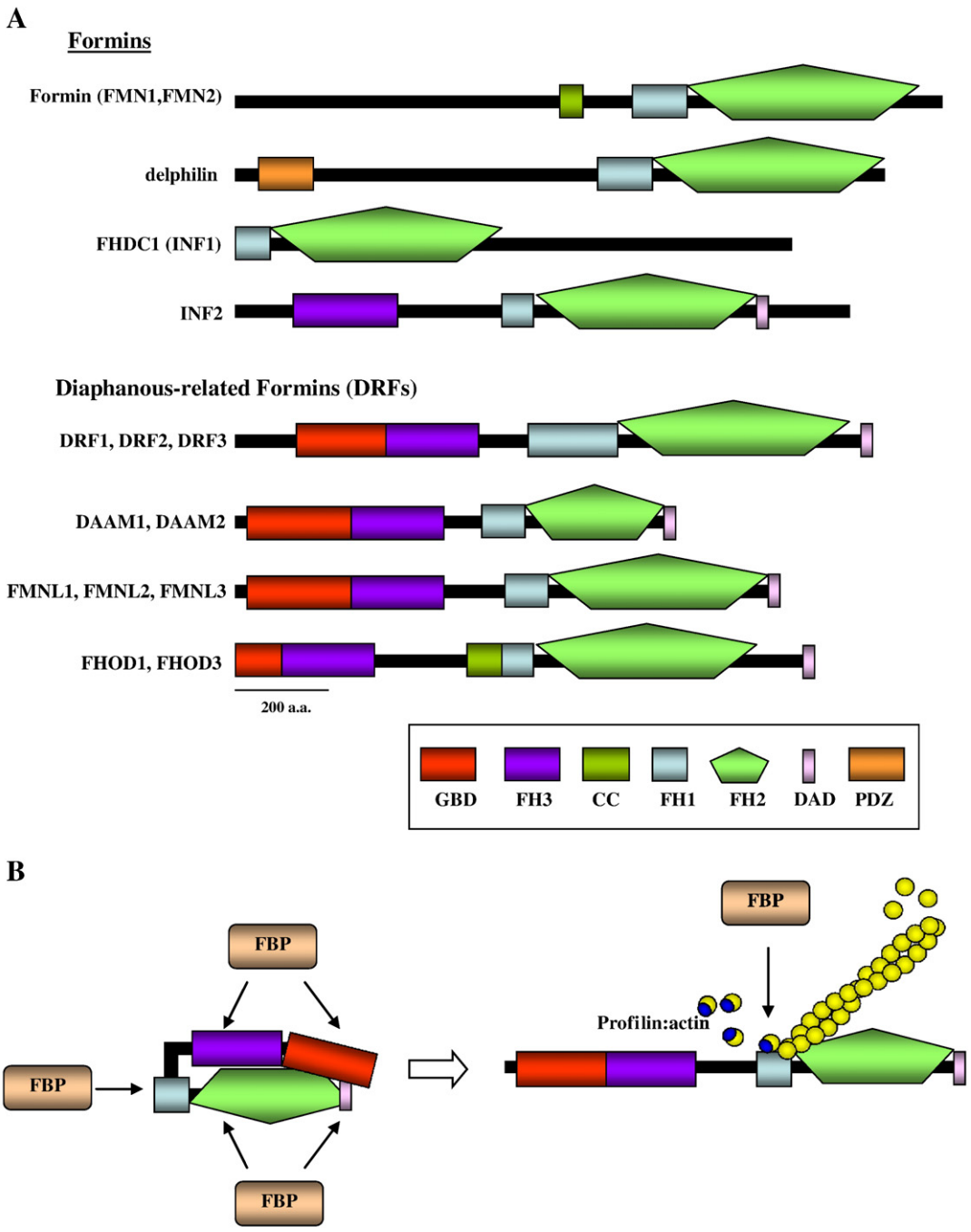

Fig. 1. A. Domain organization of the mammalian formins. The seven subgroups of the mammalian formins can be divided into formins and Diaphanous-related formins (DRFs). Representative members of all subgroups are depicted in the figure. The domain names follow the nomenclature used in the SMART data-base (http://smart.embl-heidelberg.de): GBD denotes GTPase binding domain (this domain harbors the GTPase binding domain and the Diaphanous inhibitory domain (DID), FH3 denotes formin homology domain 3 (this domain is rich in coiled-coils and it also harbors a dimerization domain), CC denotes coiled-coil domain, FH1 denotes formin homology domain, FH2 denotes formin homology domain 2, DAD denotes Diaphanous autoregulatory domain. B. Modulation of DRF activation by formin-binding proteins. Potential roles for FBPs in the regulation of the autoinhibited conformation of DRFs. Rho GTPase binding to the GBD is a major event in unlocking of the inhibitory conformation of DRF, but other FBPs can presumably serve in the process by binding to other domains of the DRFs. In the open conformation profilin interacts with the proline-rich FH1 domain and profilin:actin complexes are seeded to the FH2 actin nucleation module. FBPs can also influence the actin polymerization machinery by binding to the FH1 domain and possibly to other parts of the DRFs.

formin-binding proteins (FBPs), but many additional FBPs exist that play important roles in the regulation of formin function (Fig. 1B and Table 2). This could be accomplished by, 1), FBPs being directly involved in formin-driven actin nucleation, 2) the FBPs influencing the activated state of the formins, 3) linking formin-driven actin polymerization to Arp2/3 driven actin polymerization, or 4) influencing the subcellular localization of the formins.

While works detailing formins and their binding partners grow, it is imperative to compile the known FBPs and their effects on formins. There may not be one specific manner by which FBPs bind to formins or influence their function; however, this review will provide a comprehensive overview of the known FBPs and provide insight into some common themes of their effects on formin function. This review will only briefly mention the Rho GTPases since this will be the subject of a separate review article [this issue]. The focus of this article will be on the mammalian FBPs but it will also bring up examples from other organisms, in particular since studies in yeast and fruit fly have contributed significantly to our current understanding of the FBPs.

Table 1
Formins from selected species.

Formin name	Formin type
Saccharomyces cerevisiae	
Bni1p	DRF
Bnr1p	DRF
Schizosaccharomyces pombe	
Cdc12p	Formin
Fus1p	DRF
For3p	DRF
Dictyostelium discoideum	
ForA-H, ForJ	DRF
ForI	Formin
Arabidopsis thaliana	
AtFH1-11	Class 1 plant formin[a]
AtFH12-21	Class 2 plant formin[a]
Drosophila melanogaster	
Diaphanous	DRF
Cappuccino	Formin
FHOD	DRF
DAAM	DRF
FMNL	DRF
Formin3 (Form3)[b]	Formin
Mammals	
FMN1, FMN1	Formin
Delphilin	Formin
FHDC1 (INF1)	Formin
INF2	Formin
DRF1[c]	DRF
DRF2[c]	DRF
DRF3[c]	DRF
DAAM1-2	DRF
FMNL1-3	DRF
FHOD1, FHOD3	DRF

[a] Plants seem to lack GBD and they are probably not regulated by small GTPases. The plant formins are divided into Class 1 and Class 2 formins. Class 1 formins often contain putative membrane insertion signals, whereas Class 2 formins contain a PTEN domain.
[b] Formin3 is a *Drosophila* INF1/2 ortholog.
[c] The naming of DRF2 (=hDia2 and mDia3) and DRF3 (=hDia3 and mDia2) is rather confusing but it presumably reflects the order of characterization of the proteins in respective species. The following standard regarding the gene names are valid: *DRF1*=*DIAPH1* (human) and *DIAP1* (mouse), *DRF2*=*DIAPH2* (human) and *DIAP2* (mouse), *DRF3*=*DIAPH3* (human) and *DIAP3* (mouse).

1.1. Profilin:actin complex: fuel for the formin-driven actin polymerization machinery

Profilin was one of the first FBPs to be identified. The initial hint of a connection between profilin and formins came from a study employing a profilin-coupled affinity matrix and it was subsequently shown to have a role together with the fly formin Cappuccino during oogenesis in *Drosophila* [10,11]. These indications were confirmed from studies in *Saccharomyces cerevisiae*, which demonstrated a direct interaction between profilin and the yeast formins Bni1p and Bnr1p [12,13]. In vertebrates, there are four genes encoding profilins (*profilin I–IV*) [14]. *Profilin I* is ubiquitously expressed in most cell-types and *profilin II* also displays a rather ubiquitous expression pattern, however, it appears to be enriched in neuronal cells. In contrast, *profilins III* and *IV* have a much more restricted expression pattern; they are expressed in testis and, in the case of *profilin IV*, also in the brain. These latter profilin paralogs are quite divergent from *profilins I* and *II* in terms of their amino-acid sequence.

Profilin has a rather interesting history of identification. It was originally identified as a subunit in an inhibitor of the enzyme DNAse I [15]. The inhibitor was subsequently shown to consist of profilin and non-muscle actin in a 1:1 complex and actin conferred binding to DNAse I [15,16]. Profilin is a major actin-sequestering protein in

Table 2
Formin-binding proteins in *S. cerevisiae*, *S. pombe*, *Dictyostelium discoideum*, *Drosophila melanogaster* and mammals.

Binding partner[a]	Formin type	Reference
Saccharomyces cerevisiae[b]		
Profilin	Bni1p, Bnr1p	[12,13]
Rho1p	Bni1p	[80]
Rho3p	Bni1p	[81,87]
Rho4p	Bni1p, Bnr1p	[12]
Cdc42p	Bni1p	[13]
Bud6p	Bni1p,Bnr1p	[13,82]
Spa2p	Bni1p	[83]
Smy1p	Bnr1p	[82]
Hof1p	Bnr1p	[40]
EF1α	Bni1p	[84]
Num1p	Bni1p	[85]
Fus1p	Bni1p	[86]
Schizosaccharomyces pombe		
Profilin (Cdc3p)	Cdc12p	[88]
Rho3p	For3p	[66]
Cdc42p	For3p	[66,67]
Bud6p	For3p	[67]
Tea4p	For3p	[65,68]
Cdc15p	Cdc12p	[39]
Dictyostelium discoideum		
Rac1A	ForH (dDia2)	[89]
ProfilinII	ForH (dDia2)	[89]
VASP	ForH (dDia2)	[61]
Drosophila melanogaster		
Profilin	Cappuccino	[11]
Rho1	Cappuccino, Diaphanous	[63,90,91]
RhoA	DAAM	[43]
Spire	Cappuccino	[62,63]
Src	DAAM	[43]
Mammals		
Profilin	mDia1	[10,92]
RhoA	mDia1, mDia2, DAAM1	[41,92,93,94]
RhoB	mDia1, mDia2	[94]
RhoC	mDia1	[94]
Cdc42	mDia2 (DRF3), DAAM1	[41,93,95]
Rac1	FHOD1, FMNL1 (FRL)	[96,97,98]
RhoD	hDia2C	[38]
Rif	mDia2	[99]
VASP	mDia1	[60]
FBP17	mDia1, mDia2, DAAM1	[41]
CIP4	mDia2, DAAM1	[41]
Toca-1	mDia1, mDia2, DAAM1	[41]
Syndapin	mDia2, DAAM1	[41]
IRSp53	mDia1	[100]
Src	Formin-1, mDia2, hDia2C, DAAM1	[36,38,41,42]
Fyn	Formin-1	[42]
α-Catenin	Formin-1	[101]
DIP(AF3p21, SPIN90, WISH)	mDia1, mDia2	[44,46,47,48]
IQGAP1	mDia1	[56,57]
Dvl1-3	DAAM1, DAAM2	[76]
APC	mDia1, mDia2	[74]
EB1	mDia1, mDia2	[74]
PKCε,	PKCη mDia1	[75]
PKCζ	mDia1	[75]
PKD2	mDia1	[102]
YWK-II	mDia1	[103]
Pax6	mDia1	[104]
Glutamate receptor δ2	Delphilin	[54]
ROCK1	FHOD1	[105,106]
POPX2	mDia1	[107]
Raf-1	FHOD1	[108]
ERK	FHOD1	[108]

[a] The actin-binding capacity is a hallmark of all FH2 domains and hence of all formins and has not been included in the list (see ref. 4,5 for a review).
[b] Numerous binding partners have been identified for Bni1p and Bnr1p from various interactome approaches to identify binding partners for all *S. cerevisiae* proteins (see for instance ref. 79 and the Biogrid webpage (http://www.thebiogrid.org). Many of these potential binding partners will need to be confirmed by additional approaches and have not been included in the table.

eukaryotic cells but it does not account for the entire pool of unpolymerized actin, since the activity of other actin-sequestering proteins, such as thymosinβ4, add to the maintenance of a pool of unpolymerized actin [17]. Profilin not only sequesters monomeric actin, it also functions as a nucleotide exchange factor, facilitating the exchange of ADP for ATP on the actin monomers [18]. Profilin has three types of binding partners; actin, PIP_2 and stretches of proline-rich motifs [19,20]. The poly-L-proline binding site is separated from the actin-binding site, but there is a partial overlap between the PIP_2 and the poly-L-proline binding sites. Importantly, PIP_2 binding has been shown to interfere with the binding of profilin to actin and to proline-rich sequences [19,20]. Over 30 proteins with poly-L-proline stretches have been found to function as profilin ligands, including vasodilator-stimulated phosphoprotein (VASP), Mena and formins, and several lines of evidence indicate that profilin is a major binding partner of formins [19].

The FH1 domain is proline-rich and contains several consensus binding sites for profilin [4,5]. The number of potential profilin-binding poly-L-proline stretches varies between the different FH1 domains. The FH2 domain in most formins effectively nucleates actin filaments and formins remain bound to the barbed end of the nucleated filament [21–23]. Binding of profilin:actin complexes to the FH1 domain increases the rate of elongation considerably in a process known as processive association [23,24]. Thus, the role of profilin in formin-driven actin polymerization seems to be in the regulation of elongation speed. SH3 domain-containing proteins also bind the FH1 domain of formins; therefore, it is possible to envision that they might influence the binding of profilin. It is also possible that other profilin-binding proteins will influence the activity of profilin and thereby of formins.

1.2. FBPs and F-BAR domain-containing proteins at the interface between lipid membranes and the cytoskeleton

A major peptide screen for binding partners to the mouse formin-1, performed by Chan et al., identified a large number of FBPs [25]. This way, numerous SH3 and WW domain-containing proteins were recognized as potential formin binding partners. However, it should be noticed that rather few of them have been studied in detail with the specific object to establish if they can function as *bona fide* FBPs under physiological conditions. Up to now, FBP17 is probably the best characterized member of the original screen [25]. This protein belongs to the recently identified Fes/CIP4-homology and Bin/Amphiphysin/Rvsp (F-BAR) (also known as Pombe Cdc15 homology (PCH)) family of proteins [26,27]. FBP17 has two paralogs: Cdc42-interacting protein 4 (CIP4) and Transducer of Cdc42 activation 1 (Toca-1) and together they form the CIP4 subfamily of the F-BAR proteins [28,29]. The F-BAR proteins have emerged as critical coordinators of actin assembly and membrane dynamics [26,27,30]. The F-BAR domain is related to the classical BAR domains, which are found in endocytic proteins such as endophilin and amphiphysin [30]. In similarity to the BAR domains, the F-BAR domains form a triplet of extended α-helices, which fold into banana-shaped dimers. The concave surface of the "banana", formed by the helical dimer, can both sense and induce membrane curvature [31]. This membrane binding and deforming activity can be visualized *in vitro* and *in vivo* as the formation of extended membranous tubules [32–35]. There is a clear role for F-BAR proteins in endocytosis and several members of the F-BAR proteins bind directly to the endocytosis regulator dynamin via their SH3 domains [32–34]. Interestingly, several observations implicate roles for formins in endocytosis. mDia2 was found in endosomes in interphase cells and a splice variant of the human DRF3 (called hDia2C) binds to the endocytic Rho GTPase RhoD [36–38].

The strongest implications for a communication between formins and F-BAR proteins come from studies in *Schizosaccharomyces pombe*. It was shown that the F-BAR protein Cdc15p binds the formin Cdc12p and that both proteins are required for the organization of the contractile actomyosin ring during cytokinesis [39]. In budding yeast, the F-BAR member Hof1p binds the formin Bnr1p in a manner dependent on the activation of Rho4p and that Hof1p and Bnr1p are acting in a pathway that regulates cytokinesis [40]. Studies in mammalian systems are scarce, but it has been found that the SH3 domains of FBP17, CIP4 and Toca-1 can mediate the interaction to mDia1, mDia2 and DAAM1 [41]. CIP4 was furthermore proposed to collaborate with DAAM1 and Src in the formation of filopodia [41].

The F-BAR proteins are also involved in the regulation of actin dynamics via their interconnection to the WASP/N-WASP:Arp2/3 actin polymerization module [3,29,35]. In this way the F-BAR protein can bridge the formin-driven and Arp2/3-driven actin polymerization machineries as seen in the example of the *S. pombe* Cdc15p [39]. In resting cells, WASP proteins are predominantly folded in an autoinhibitory conformation, thereby masking the binding site to the Arp2/3 complex, which is present at the WASP C-terminus [3]. A series of events, involving the concerted binding of phosphoinositides and Rho GTPases, as well as alterations in the phosphorylation status of WASP, results in the release of the autoinhibited conformation of WASP [3]. Several F-BAR proteins bind the WASP family of proteins and the role of Toca-1 has been established in a study by Ho et al. (Fig. 2) [29]. In non-stimulated cells, a majority of the N-WASP appears to be sequestered by members of the WASP interacting protein (WIP) family of proteins in an autoinhibited conformation. Cell signaling induces a dissociation of the WIP:N-WASP complex and Toca-1 was proposed to have a role in this dissociation since it increases the N-WASP:Arp2/3-driven actin polymerization *in vitro* [29]. The exact mechanism underlying this effect is, however, not clear. It is currently not known if the F-BAR proteins can have a similar role in the regulation of formin-driven actin polymerization but this possibility remains an attractive possibility.

1.3. Src family kinases are modulators of formin function

Src family kinases constitute an important group of FBPs. These non-receptor tyrosine kinases have multiple roles in the regulation of formin function, since they not only bind formins directly but also modulate the function of other FBPs. Using a panel of GST-SH3 domains derived from various signaling proteins, Uetz et al. found that formin-1 could bind Fyn and Src [42]. Furthermore, Src was shown to relocate formin-1, which is normally a nuclear protein, to the plasma membrane. Tominaga et al. used a similar approach to identify Src as a binding partner to mDia2 [36]. The authors noticed that mDia1 and mDia2 colocalize with activated Src in the cleavage furrow of dividing cells and in endosomes in interphase cells suggesting a role for formins in endosomal trafficking. In addition, Src was needed for mDia2-mediated cellular effects such as formation of stress fibers and activation of serum response element [36]. Moreover, a yeast two-hybrid system screen identified a splice form of human DRF3 (also known as hDia2C) as a RhoD-binding protein [38]. Interestingly, this isoform lacked the RhoA and Cdc42 binding sites present in the normal splice variant of DRF3, instead it contained a RhoD-binding motif which is not found in the normal DRF3. RhoD has a role in the regulation of endosome trafficking and DRF3 was shown to be needed in this process. This effect was dependent on Src, and the DRF3 splice variant directly affected Src activation [38].

Yet another member of the DRFs, DAAM1, has been shown to bind Src, and Src family kinases were needed for the DAAM1-dependent formation of filopodia [41]. This notion was further supported by a study in *Drosophila*, which demonstrated an interaction between fly DAAM and Src. In this case, Src was acting downstream of DAAM to organize the actin filaments of the fly tracheal system [43]. Finally, Src has been found to influence the activity of other FBPs, such as mDia-interacting protein (DIP) [44]. This study showed that EGF stimulation resulted in phosphorylation of DIP in a manner dependent on Src (see

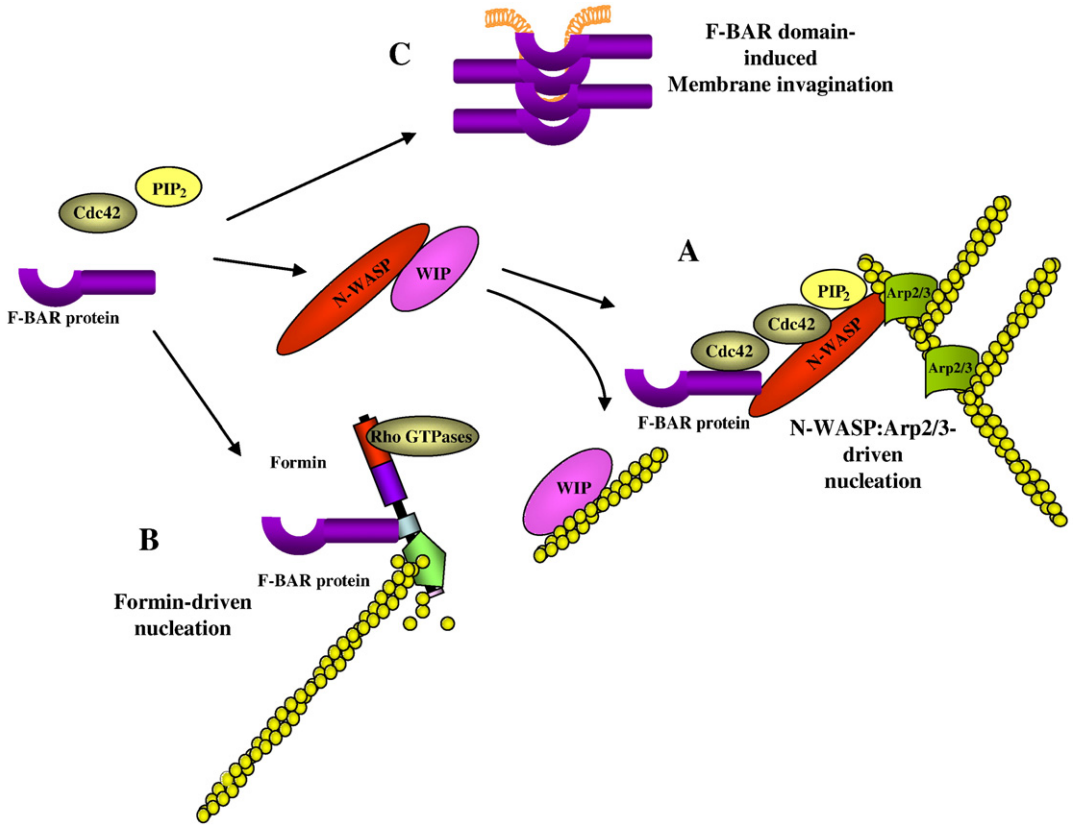

Fig. 2. FBPs are multi-subunit proteins bridging actin polymerization and membrane trafficking. A. F-BAR proteins (for instance Toca-1) activate the N-WASP:Arp2/3 polymerization machinery by unlocking the inactive WIP:N-WASP complex. This cue requires the concerted action of Toca-1, activated Cdc42 and PIP_2 and leads to the formation of Arp2/3 dependent branched actin filaments. B. F-BAR proteins (such as Cdc15p or Toca-1) can also bind formins and could possibly modulate the formin-driven actin polymerization. C. Moreover, the F-BAR domains can bind lipid membranes and induce a curvature of the membranes. This leads to invagination of the plasma membrane and the appearance of tubular structures. Thus, F-BAR proteins can function to coordinate the actin polymerization and membrane trafficking events.

further next chapter). DIP, in turn, bound p190RhoGAP and Vav2, and DIP phosphorylation was required for phosphorylation and activation of these two guanine nucleotide exchange factors for Rho family members [45].

1.4. Dia-interacting protein in actin regulation

DIP has been cloned independently by several laboratories; it was first identified as *AF3p21* (ALL1-fused gene from chromosome 3p23), a *MLL* fusion partner in a patient with leukemia [46], and later as an Nck-binding protein called SPIN90 (SH3 protein interacting with Nck, 90 kDa) [47], Grb2 and N-WASP-binding protein called WISH (WASP interacting SH3 protein) [48] and Dia-interacting protein (DIP) [44]. The ascribed role of DIP in regulation of actin reorganization differs with the point of view taken by the individual research group. Research groups working on WASP-dependent actin regulation tend to favor a role for DIP in modulation of WASP/Arp2/3-dependent actin polymerization [48], whereas researchers working on formins tend to favor a role for DIP in Dia-dependent actin polymerization [44,45]. Collectively, these studies suggest a role for DIP as a bridge between formin-dependent and Arp2/3-dependent actin polymerization. In the form of WISH, DIP was found to be an effective activator of N-WASP and N-WASP:Arp2/3 driven actin polymerization and ectopic expression of WISH and N-WASP induced Cdc42-independent formation of filopodia. However, WISH did not induce Arp2/3-driven actin polymerization on its own [48]. In contrast, DIP in the guise of SPIN90 was shown to directly bind G-actin and the Arp2/3 complex via its C-terminus. Moreover, a C-terminal fragment of SPIN90 effectively induced actin polymerization *in vitro* in the presence of the Arp2/3 complex. SPIN90 also induces actin polymerization seen as the formation of actin comet tails [49]. The apparent discrepancy between these two studies is presumably dependent on the differences in experimental design. The former study employed a full-length DIP but the latter study used a C-terminal fragment of DIP [48,49]. The domains present in this part of the protein could very well be masked in the full-length protein.

Although DIP has an important role in N-WASP and Arp2/3 dependent actin polymerization, it is clear that DIP also is needed for DRF-mediated actin polymerization. As already mentioned, DIP is needed for the EGF-induced phosphorylation of p190RhoGAP and Vav2 [45]. EGF stimulation leads to DIP-dependent downshift in the Rho activation in favor of an increased Rac1 activation. DIP over-expression resulted in a delayed recovery of stress fibers after EGF stimulation and this effect was dependent on an ability of DIP to trigger membrane targeting of p190RhoGAP and Vav2 [45]. Knock down of DIP expression with siRNA resulted in a decreased membrane targeting of p190RhoGAP and Vav2, and decreased cell migration. Thus, the DIP communication with p190RhoGAP and Vav2 appeared to be necessary for the acute effects on the actin reorganization. A study by Eisenmann et al. demonstrated that the DIP SH3 domain bound the FH1 domain of DRF and the DIP leucine-rich region was found to be involved in regulation of FH2-driven actin polymerization [50]. DIP has also been proposed to have a role in endocytosis and it binds proteins that are involved in clathrin-mediated endocytosis, such as dynamin and syndapin [51,52]. Moreover, DIP was shown to have a specific role in neuronal cells in the formation of dendritic spines in an N-WASP independent manner [53]. This effect might be unique for the DIP:N-WASP collaboration since, so far, DRFs have not been implicated to be involved in the regulation of dendritic spines. It is possible that DIP could collaborate with the glutamate receptor δ2-binding formin,

delphilin, which has been found to be localized to dendritic spines [54]. This hypothesis will require further investigations, however. Clearly, DIP is an important molecule that affects actin polymerization via many different binding partners.

1.5. IQGAPs: a bridge between formins and the Arp2/3 complex

There are an increasing number of examples of communication between the different protein complexes needed for actin nucleation. It has already been discussed that F-BAR proteins and DIP can bridge the Arp2/3-driven and the formin-driven actin nucleation machineries [29,39,40,45,48]. IQGAPs are yet another example of a multi domain protein that binds many partners including Cdc42 and Rac1 [55]. There are three IQGAP paralogs in human, but up to now, the best studied of them is IQGAP1. This protein localizes to cellular compartments that undergo dynamic actin reorganization, such as lamellipodia and cell–cell adhesion structures [55]. IQGAP1 can directly bind to actin filaments and induce bundling of them, but another function of the protein may be to induce the specific subcellular localization of the actin polymerization machineries, since IQGAP1 was shown to bind N-WASP:Arp2/3 and mDia1 [55–58]. Interestingly, IQGAP1 appears to be able to bind N-WASP and mDia1 simultaneously. The C-terminal part of IQGAP1 mediates the interaction to mDia1, whereas the N-terminal portion of IQGAP1 seems to trigger N-WASP:Arp2/3-driven actin polymerization. There are conflicting data, however, regarding the exact position of the binding sites (Fig. 3) [56–58]. Lamellipodia are formed by branched actin filaments as well as bundles of actin filaments. Since IQGAPs possess the ability to bind to both N-WASP:Arp2/3 and the formins, they could provide the means for the formation of both types of actin organizations.

1.6. Communication between actin nucleators

Some profilin-binding proteins also bind directly to formins. For example, mDia1 was found to bind the profilin ligand VASP. VASP is a protein proposed to associate with the barbed ends of actin filaments. It serves to protect them from capping-proteins and to support the formation of filopodia [59]. mDia1 was not only found to interact directly with VASP, both proteins turned out to function cooperatively to induce actin polymerization and serum response element (SRE) dependent transcription [60]. In the former study the VASP-binding domain in mDia1 was not unambiguously identified. While the FH2 domain appeared important, the interaction apparently required additional domains [60]. The importance of the FH2 domain for the interaction was supported by observations in the slime mould *Dictyostelium*. DdVASP was shown to bind dDia2 (ForH) via this domain and the formation of filopodia required both proteins [61]. It was furthermore observed, however, that the localization of DdVASP and dDia2 to filopodia tips can occur independently of one another [61].

The final example of connections between different types of actin nucleators is the communication between formins and Spir. Studies in *Drosophila* have demonstrated a genetic interaction between Cappuccino (Capu) and Spir in the regulation of cytoplasm streaming in the oocyte, a process that requires actin filaments and microtubules (MTs) [62]. Interestingly, a direct interaction was demonstrated to occur via the FH2 domain of Capu and the N-terminal domain containing kinase noncatalytic C-lobe (KIND) and WH2 domains on Spir. In the initial study, the interaction was not found to affect the nucleation ability of either Capu or Spir, however, the interaction interfered with the FH2-dependent cross-linking of actin filaments and MTs [62]. Importantly, Rho1-binding was found to restore the cross-linking ability of Capu, implicating a regulatory role of Rho1. A later study reported that the binding between Capu and Spir is conserved from insects to mammalian systems. In addition, it showed that the KIND domain of Spir blocks the FH2-driven actin nucleation and leads to an increased actin nucleation by Spir, suggesting that Spir and formins cooperating to induce actin nucleation is a general phenomenon in many different organisms [63].

1.7. Formins in the regulation of cell polarity and microtubule stability

In *S. pombe*, MTs are necessary for a proper regulation of cell shape and they also provide a cue to establish cell polarity after the completion of cytokinesis [64]. After cell division the plus ends of the MTs grow to the tip of the expanding daughter cell until they reach the distal cortex of the cell. The MTs transport a protein complex consisting of Tea1p and Tea4p to the tip of the MTs and deposit them to the cortex [65]. This protein complex is responsible for establishment of cell polarity and Tea4p thereafter attracts the formin For3p to the cell cortex. At the cortex, For3p is activated and can trigger the formation of actin filament bundles [65,66]. The MT-dependent process seems to be the critical activating cue, but For3p

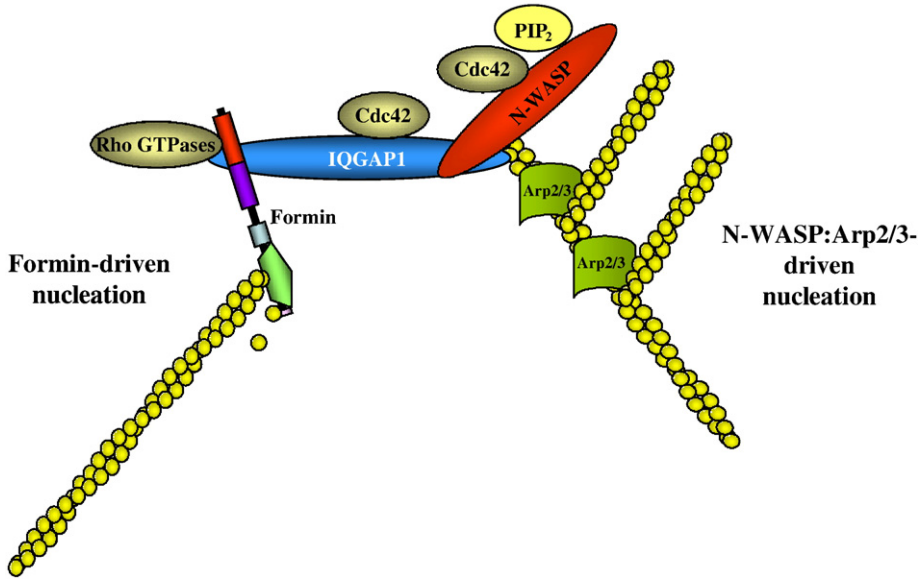

Fig. 3. Bridging the formin-dependent and N-WASP:Arp2/3-dependent actin polymerization machineries. Several FBPs have potential roles in bridging the formin-driven and Arp2/3-driven actin polymerization machineries. IQGAP1 is possibly the best studied example and it can bind formins and N-WASP:Arp2/3 via different interaction modules.

binds additional proteins at the cell cortex, which are important for the modulation of the For3p activation [65–68].

In mammalian cells, DRFs, most notably mDia1 and mDia2, have been ascribed roles in pathways that regulate MT stability and MT organizing center (MTOC) orientation and, thereby, in pathways that regulate directed cell migration [69–71]. In eukaryotic cells, there are two pools of MTs; one pool that is under a rapid and dynamic reorganization, so-called dynamic instability, and one pool that constitutes stable MTs [72]. The formation of stable MTs is associated with a removal of a C-terminal tyrosine residue by a carboxyl peptidase. The MT stabilization is further maintained by additional post-translational modifications, e.g. acetylation [72]. The G-protein coupled lysophosphatidic acid (LPA) receptor has been shown to signal to stable MTs via a pathway that involves the activation of RhoA and mDia1 and/or mDia2 [73]. The formation of stable MTs is further dependent on the MT-binding proteins EB1 and APC [74]. Interestingly, mDia2 was shown to bind to both proteins allowing the formation of a trimeric complex. Moreover, LPA was shown to result in phosphorylation of GSK3β on serine 9 and the activity of this Ser/Thr protein kinase was necessary for the formation of stable MTs. The Ser9 phosphorylation was modulated by the activity of the so-called novel PKCs (PKCε and PKCη) [75]. Interestingly, mDia2 was shown to directly bind PKCε and PKCη and mediate the GSK3β phosphorylation. Since mDia binds the downstream effectors for GSK3β, it is conceivable that mDia can function as an adapter, bringing together several of the components needed for the regulation of MT stability. While the detailed topology of the interactions will need further analysis, it is easily observed that the MT stabilization pathway involves multiple formin-binding proteins.

1.8. FBPs in the Wnt signaling pathway

A yeast two-hybrid screen isolated the Disheveled-associated activator of morphogenesis (DAAM1 and DAAM2) as Dvl-binding proteins [76]. This finding raised some interests since Dishevelled (Dvl) is an adaptor protein downstream of the Wnt receptor Frizzled and, thus, the data suggested a link between Wnt signaling and formins. In humans, there are three Dishevelled paralogs: Dvl1–3 [77]. In vertebrates, Wnt-Frizzled signals to stabilization of β-catenin via the N-terminal DIX domain. In the absence of Wnt, β-catenin is phosphorylated by the axin/APC/GSK3β complex. This phosphorylation targets β-catenin for ubiquitinylation and proteasomal degradation [78]. Wnt stimulation results in targeting of axin/APC/GSK3β to the DIX domain and as a result, β-catenin evades the proteasome and is set free to enter the nucleus and induce transcription of specific genes. This pathway is known as the “canonical” Wnt/β-catenin pathway. In addition, Wnt-Frizzled also induces planar cell polarity, through a “non-canonical” pathway and DAAM1 was suggested to be involved in this pathway. The interaction is mediated between the PDZ domain of Dvl and a peptide at the extreme C-terminus of DAAM. Initial information implicated that DAAM could activate RhoA in a RhoGEF-dependent manner [76]. However, this model is controversial since it contradicts the well established role of DRFs as downstream effectors for Rho GTPases [4,5] At least two recent studies, one in mammalian cells and one in *Drosophila*, support a function for DAAM as a binding partner for Rho GTPases rather than an upstream activator [41,43]. Surprisingly, *Drosophila* DAAM does not seem to bind dishevelled and thus DAAM has no clear role in planar cell polarity in *Drosophila*. Instead it was found to be needed for the organization of actin cables in special cells during the development of the tracheal network [43].

1.9. Concluding remarks

The number of identified FBPs has increased steadily during recent years. Different proteomic approaches have created interactomes of all yeast proteins leading to the identification of dozens of potential yeast FBPs. However, a majority of the interactors will need confirmation by independent studies in order to establish if they are *bona fide* FBPs. The detailed picture of each FBP might be lacking, but some common themes have emerged. Although formins can catalyze actin nucleation by virtue of their FH2 domains, FBPs are required for the regulation of the process *in vivo*. Moreover, FBPs have turned out to bring together different types of actin polymerization machineries and this ability is presumably critical for the production of different organizations of actin filaments, e.g. at the leading edge of migrating cells. Therefore, an increased focus on the FBPs is likely to give important insights into the complex signaling networks that coordinate actin assembly.

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